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Robust Summary - Polyethylbenzene Bottoms Stream

Freezing Point

Test Substance:	Polyethylbenzene Bottoms Stream (PEB) is 100% of the complex mixture CAS RN. 68987-42-8. PEB is a coproduct of ethylbenzene manufacture and a Class II complex mixture consisting of various isomers of alkylbenzene and diphenyl hydrocarbons.
Method/Guideline:	OECD #102 (1995)
Type (test type):	ASTM D 1015-99
GLP:	No
Year (study performed):	2005
Test Conditions:	The PEB sample was a blend of equal volumes of six PEB samples from different suppliers. The PEB sample was prepared at ABC Laboratories, Inc., Columbia, MO. The freezing point testing was performed at Harris Testing Laboratories, Houston, TX. The freezing point of the test substance was determined in triplicate following ASTM method D 1015-99. As the test substance was cooled, the temperature was recorded every fifteen seconds. A temperature versus time plot was prepared for each replicate determination. The freezing point was determined from the equilibrium portion of the freezing curve.
Results:	Analysis of the equilibrium portion of each replicate resulted in a test substance freezing point of –58.8°C. The results are summarized below: Replicate / Freezing Temperature (°C) 1 / -58.8 2 / -58.8 3 / -58.8 Mean / -58.8 ± 0.0
Conclusion: (Laboratory contractor)	The freezing point of PEB was determined to be –58.8 ± 0.0°C.
Reliability:	**2.** Reliable with restrictions. The testing laboratory is a reputable analytic laboratory but does not meet all procedures specified under GLP.
Reference:	Determination of Freezing Point for a Polyethylbenzene Bottoms Stream (PEB) Blend. 2005. Huntley, K. ABC Study No. 49022, ABC Laboratories, Inc. Columbia, MO. Sponsor American Chemistry Council, Arlington, VA ASTM Method D1015-99, Standard Test Method for Freezing Points of High Purity Hydrocarbons. 11pp.
Other (source) Last changed	1/31/06

Robust Summary - Polyethylbenzene Bottoms Stream

Vapor Pressure

Test Substance:	Polyethylbenzene Bottoms Stream (PEB) is 100% of the complex mixture CAS RN. 68987-42-8. PEB is a coproduct of ethylbenzene manufacture and a Class II complex mixture consisting of various isomers of alkylbenzene and diphenyl hydrocarbons.
Method/Guideline:	OECD #104 (1995)
Type (test type):	Vapor Pressure determination
GLP:	Yes
Year (study performed):	2005
Test Conditions:	The PEB sample was a blend of equal volumes of six PEB samples from different suppliers. Reagent water had been purified using a Millipore Milli-Q Purification system. Thermometer was NIST-verified. The vapor pressure apparatus was a Terranova model 908A dual capacitance diaphragm gauge controller, Baratron pressure transducer, Franklin electric vacuum pump model 4401007400, and 100-mL long-necked, round bottom flasks with sidearm. Atmospheric pressure was checked prior to use each day using a NOVA mercury barometer. Verification of the vapor pressure testing apparatus was performed once a year by determining the vapor pressure of water at 20^{0}C in triplicate. The vapor pressure of water was determined to be 17.4 ± 0.1 torr (2320Pa) within 0.4% of reported literature values. At the initiation of the study, approximately 25ml PEB was added to the test flask. The sample was degassed at reduced temperature by supercooling using an acetone/dry ice bath. The flask valve was opened for several minutes to remove any liberated air, then was closed. Following 30 minutes of immersion in the water bath set at 10°C, the vapor pressure reading was recorded. The temperature of the waterbath was adjusted to 20 and 30°C. After allowing the sample to equilibrate to each test temperature for 30 minutes, the vapor pressure value was recorded. The temperature of the waterbath at each test temperature was verified using a thermometer. This procedure was repeated for a second replicate determination.
Results:	The vapor pressure of PEB was determined to be less than 90 Pa at 10, 20, and 30°C, respectively. All pressure readings at 10, 20, and 30°C were less than 0.7 torr (90 Pa). (see table below) The vapor pressure was reported as less than 10^2 Pa at each of the temperatures evaluated.
Conclusion: (Laboratory contractor)	The vapor pressure of PEB was determined to be less than 10^2 Pa at 10, 20, and 30°C, respectively.
Reliability:	**1.** Reliable without restrictions.

Target Temp. (°C)	Replicate Number	Temp. Reading (°C)	Vapor Pressure (torr)[a]	Vapor Pressure (Pa)[1]
10	1	10.0	< 0.7	< 90
	2	10.0	< 0.7	< 90
20	1	20.0	< 0.7	< 90
	2	20.0	< 0.7	< 90
30	1	30.1	< 0.7	< 90
	2	30.0	< 0.7	< 90

[a]1 torr = 1.33322 x 10^2 Pa

Reference:	Determination of Vapor Pressure for a Polyethylbenzene Bottoms Stream (PEB) Blend. 2005. Huntley, K. ABC Study No. 49024, ABC Laboratories, Inc. Columbia, MO. Sponsor American Chemistry Council, Arlington, VA
Other (source) Last changed	1/31/06

Robust Summary - Polyethylbenzene Bottoms Stream

Boiling Point -measured

<table>
<tr><td>Test Substance:</td><td>Polyethylbenzene Bottoms Stream (PEB) is 100% of the complex mixture CAS RN. 68987-42-8. PEB is a coproduct of ethylbenzene manufacture and a Class II complex mixture consisting of various isomers of alkylbenzene and diphenyl hydrocarbons.</td></tr>
<tr><td>Method/Guideline:</td><td>OECD #103 (1995)</td></tr>
<tr><td>Type (test type):</td><td>Automated system, improved Siwoboloff method</td></tr>
<tr><td>GLP:</td><td>Yes</td></tr>
<tr><td>Year (study performed):</td><td>2005</td></tr>
<tr><td>Test Conditions:</td><td>The PEB sample was a blend of equal volumes of six PEB samples from different suppliers. A Mettler FP900 Thermosystem consisting of a Mettler FP81HT MBC Cell attached to a Mettler FP90 Central processor was used to determine the boiling point of the test substance. A Princo mercury barometer was used for barometric pressure measurements. To verify that the instrument was working properly, the boiling point of ethyl alcohol was determined to be 78.5+0.1^{0}C, very similar to the CRC Handbook value of 78.5^{0}C.
PEB was added to a boiling point tube to a height of 15-18 mm. A boiling capillary was inserted into the boiling point tube until the capillary rested on the base of the tube. The tube was analyzed by inserting the tube into the center slot of the instrument. This sample was analyzed starting at 258°C and increasing at +0.2°C/minute until the boiling point was reached. The boiling point recorded was calculated by the instrument using the actual boiling temperatures and barometric pressure (99.2 kPa) measurements. The boiling point values were corrected to standard pressure (101.325 kPa) automatically by the instrument.</td></tr>
<tr><td>Results:</td><td>The boiling point of PEB was determined to be 262.2 ± 0.3°C (535.4 K) as shown in the following table:
<table>
<tr><th>Replicate</th><th>Boiling Temperature (°C)</th><th>Boiling Point (°C)</th></tr>
<tr><td>1</td><td>260.9</td><td>261.9</td></tr>
<tr><td>2</td><td>261.3</td><td>262.3</td></tr>
<tr><td>3</td><td>261.5</td><td>262.5</td></tr>
<tr><td></td><td>Mean</td><td>262.2 ± 0.3
(535.4 K)</td></tr>
</table>
There was no indication of test substance decomposition.</td></tr>
<tr><td>Conclusion:
(Laboratory contractor)</td><td>The boiling point of PEB was determined to be 262.2 ± 0.3°C (535.4 K).</td></tr>
<tr><td>Reliability:</td><td>2. Reliable with restrictions. Since PEB is a complex mixture of hydrocarbons, a boiling point range was modeled using EPIWIN computer model, V3.12 (U.S. EPA, 2000). For the principal chemical components in PEB, modeled boiling point values ranged from 191 to 291°C.</td></tr>
<tr><td>Reference:</td><td>Determination of Boiling Point for a Polyethylbenzene Bottoms Stream (PEB) Blend. 2005. Huntley, K. ABC Study No. 49023, ABC Laboratories, Inc. Columbia, MO. Sponsor American Chemistry Council, Arlington, VA
U.S. EPA. 2000. API (Estimation programs interface) suite, V 3.12, subroutine MPBPWIN, V 1.41. US Environmental Protection Agency, Office of pollution prevention and toxics, Washington DC.</td></tr>
</table>

Other (source) Last changed	1/31/06

Robust Summary - Polyethylbenzene Bottoms Stream

Boiling Point- modeled

Test Substance	Polyethylbenzene Bottoms Stream (PEB) is 100% of the complex mixture CAS RN. 68987-42-8. PEB is a coproduct of ethylbenzene manufacture and a Class II complex mixture consisting of various isomers of alkylbenzene and diphenyl hydrocarbons.
Method/Guideline	EPIWIN computer model; V3.12 (U.S. EPA, 2000). This model calculates boiling point based on the method of Stein and Brown (J. Chem. Inf. Comput. Sci. 34, 1994).
GLP	No
Year (study performed)	Not Applicable
Results: Boiling Point Value	Calculated and measured boiling point data for representative constituents of PEB are listed below. The data identify a potential boiling point range for substances represented by CAS RN. 68987-42-8.

Chemical Name	Boiling Point, °C Measured	 Modeled
Diethylbenzene	181.0	191
Cyclohexylbenzene	240.1	238
1,2,5-triethylbenzene	215.9	230
1,2,4-triethylbenzene	218.0	230
Diphenylmethane	265.0	269
1,1-diphenylethane	272.6	276
1,2-diphenylethane	284.0	285
1,1'-diphenylpropane	281.6	291

	Measured values for the respective compounds were cited by the EPIWIN experimental database.
Pressure, units	Not Applicable
Decomposition	Not Applicable
Remarks	Values given above represent a range of estimated and measured boiling point determinations for the principal chemical components characterized in PEB (CAS RN. 68987-42-8).
Conclusions	For the principal chemical components characterized in PEB (CAS RN No. 68987-42-8), modeled boiling point values ranged from 191 to 291°C. Measured boiling point values for these constituents cited in EPIWIN's experimental database ranged from 181 to 284 °C.
Reliability	1. Reliable without restrictions. This robust summary presents measured and modeled boiling point ranges based on a characterized PEB stream.
References	U.S. EPA. 2000. API (Estimation programs interface) suite, V 3.12, subroutine MPBPWIN, V 1.41. US Environmental Protection Agency, Office of pollution prevention and toxics, Washington DC.
Other (source) Last changed	 1/31/06

Robust Summary - Polyethylbenzene Bottoms Stream

Partition Coefficient

Test Substance	Polyethylbenzene Bottoms Stream (PEB) is 100% of the complex mixture CAS RN. 68987-42-8. PEB is a coproduct of ethylbenzene manufacture and a Class II complex mixture consisting of various isomers of alkylbenzene and diphenyl hydrocarbons.
Method/guideline	OECD Method 117, HPLC method (2004)
GLP	Yes
Year (study performed)	2005
Test Conditions:	The PEB sample was a blend of equal volumes of six PEB samples from different suppliers. The HPLC system included a Phenomenex Primesphere 5 C18 HC column, 250 mm x 4.6 mm id, with a mobile phase of 75:25 acetonitrile:reagent water at a flow rate of 1.0 mL/min. Fifty microliter samples of a 11.5 µg/mL solution of PEB in mobile phase were injected, and the emergence of the material was observed using UV detection (λ = 210 nm). Eight reference compounds were used to generate the linear relationship between log k (k = capacity factor) and log Pow. Using the HPLC retention time for the peak of the test substance, the log k was determined, and the log Pow value was calculated using a linear equation developed from the reference compounds. HPLC analysis of the test substance resulted in multiple peaks, thirteen of which were attributed to PEB. The log P_{ow} values for each of the peaks of the test substance were determined by substituting their experimentally determined log k values into the equation derived from the log k versus log P_{ow} graph constructed from the reference standards.
Results: Log Pow Temperature, °C Remarks	 4.08 to 6.01 20 °C The cited values represent a range of Log Pow values for components making up the complex mixture of PEB.
Conclusion: (Laboratory contractor)	Log Pow = 4.08 to 6.01
Reliability:	**1.** Reliable without restrictions.
Reference	Serak, Kelda. 2005. Determination of n-Octanol/Water Partition Coefficient(s) for Polyethylbenzene Bottoms Stream Blend (PEB Blend) by High Performance Liquid Chromatography (HPLC). ABC Laboratories, Inc., Columbia, MO. Sponsor: American Chemistry Council, Arlington, VA
Other (source) Last changed	 1/31/06

Robust Summary - Polyethylbenzene Bottoms Stream

Water Solubility

Test Substance	Polyethylbenzene Bottoms Stream (PEB) is 100% of the complex mixture CAS RN. 68987-42-8. PEB is a coproduct of ethylbenzene manufacture and a Class II complex mixture consisting of various isomers of alkylbenzene and diphenyl hydrocarbons.
Method/Guideline	OECD Method 105 (1995)
GLP	Yes
Year (study performed)	2005
Test Conditions:	The PEB sample was a blend of equal volumes of six PEB samples from different suppliers. Water solubility was measured using the shake flask method described in OECD guideline 105 and the Official Journal of the European Communities (OJ). Test samples were prepared by adding 3 mL of PEB to each of three, 40-mL plastic centrifuge tubes. Thirty-three milliliters of reagent water was added to each tube. The samples were capped and placed on an orbital shaker water bath set at 30 °C and agitated. One replicate was removed from the shaker after approximately 24, 48, and 72 hours and placed on a shaker at 20 °C. Five days after placing the first sample on the shaker at 20 °C, the three samples were removed. Samples were centrifuged for 30 minutes at 20,000 rpm (44,720 x g) and 20 °C. The aqueous layers were removed to 40-mL scintillation vials using glass syringes with removable needles. Twenty mL of each sample was extracted and analyzed by gas chromatography. Analyses were done using gas chromatography with a flame ionization detector. Responses of standards and samples were calculated as the sum of the responses from six marker peaks within the PEB chromatogram.
Results Value, at temperature °C Description pH value pKa value at 25 °C Remarks	 29.5 ± 1.4 mg/L at 20 ± 0.5°C N/A 8.04, 7.10, and 7.24 at the 24-, 48-, and 72-hour sampling points, respectively N/A The solubility measurements at 48 and 96 hours averaged 30.7 mg/L and 28.4 mg/L, respectively. The final water solubility value was the overall mean of the 48 and 96 hour sample determinations.
Conclusion: (Laboratory contractor)	Water solubility was 29.5 ± 1.4 mg/L at 20 ± 0.5°C
Reliability:	**1.** Reliable without restrictions.
Reference	Serak, Kelda. "Determination of Water Solubility for Polyethylbenzene Bottoms Stream Blend (PEB Blend)." ABC Laboratories, Inc., Columbia, MO. Sponsor: American Chemistry Council, Arlington, VA
Other (source) Last changed	 1/31/06

Robust Summary - Polyethylbenzene Bottoms Stream

Direct Photodegradation

Test Substance:	Polyethylbenzene Bottoms Stream (PEB) is 100% of the complex mixture CAS RN. 68987-42-8. PEB is a coproduct of ethylbenzene manufacture and a Class II complex mixture consisting of various isomers of alkylbenzene and diphenyl hydrocarbons.
Method/Guideline:	Other: Technical discussion
GLP	Not applicable
Year (study performed):	Not applicable
Type (air, soil, water, other):	Water
Test Substance: **[components]**	Polyethylbenzene Bottoms (PEB, CAS RN 68987-42-8) is a co-product of ethylbenzene manufacture and a Class II complex mixture. It consists of various isomers of the following principal components. • Diphenylethanes • Diphenylmethanes • Triethylbenzenes • Diphenylpropanes
Light Source:	Not Applicable
Light Spectrum: • Wave length value (upper/lower)	Not Applicable
Relative Intensity:	Not Applicable
Test Substance Spectrum:	Not Applicable
Test Conditions: • Note: Concentration, temperature, test system type, replication, deviations from guideline or protocol	Not Applicable
Direct Photolysis: • Results: half-life, % degradation, quantum yield	Not Applicable
Indirect Photolysis: • Results: type of sensitizer, concentration of sensitizer, rate constant, % degradation, half-life	Not Applicable
Degradation Products: • Note: Identification, concentration	Not Applicable

Conclusion:	**Technical Summary of Direct Photolysis** Direct photolysis of an organic molecule occurs when it absorbs sufficient light energy to result in a structural transformation (Harris, 1982). If the absorbed energy is high enough, the resultant excited state of the chemical may transform to a different structure. A prerequisite for direct photodegradation is the ability of one or more bonds within a chemical to absorb ultraviolet (UV)/visible light in the 290 nm to 750 nm range. Light wavelengths longer than 750 nm do not contain sufficient energy to break chemical bonds, while wavelengths below 290 nm are shielded from the earth by the stratospheric ozone layer (Harris, 1982). The majority of the constituents identified in PEB consist of various isomers of alkylbenzene and diphenyl structures. Harris (1982) notes that single ring aromatics do not absorb sufficient light energy above 290 nm to cause photolysis. Therefore, those types of constituents are not subject to photolysis. Similarly, diphenyl structures tend not to display absorbance maxima within the 290 – 750 nm range. Characteristic absorbance maximum (λ_{max}) and molar extinction coefficients (ε) for three compounds, which were identified as components in PEB are shown below. Other constituents in PEB would have absorbance maxima and extinction coefficients in the range of those chemical.

	λ below 290 nm		**λ above 290 nm**	
Hydrocarbon	λ_{max}	ε	λ_{max}	ε
Cyclohexylbenzene	260	200	--	--
Diphenylmethane	260	470	--	--
1,2-diphenylethane	214	13,300	295	3000

Data from NIST Chemistry WebBook (http://webbook.nist.gov/chemistry)

Overall, this category of substances will not demonstrate a significant extent of degradation resulting from direct photolysis.

Reliability:	**1.** Reliable with restrictions. The technical summary presented herein was based on a well-regarded scientific handbook and reference database.
Reference:	National Institute of Standards and Technology (NIST). 2003. NIST Standard Reference Database Number 69 – March 2003 Release. NIST Chemistry WebBook. http://webbook.nist.gov/chemistry Harris, J.C. 1982. Rate of Aqueous Photolysis, Chapter 8 in: W.J. Lyman, W.F. Reehl, and D.H. Rosenblatt, eds., Handbook of Chemical Property Estimation Methods, McGraw-Hill Book Company, New York, USA.
Other (source): Last changed	1/31/06

Robust Summary - Polyethylbenzene Bottoms Stream

Indirect Photodegradation

Test Substance:	Polyethylbenzene Bottoms Stream (PEB) is 100% of the complex mixture CAS RN. 68987-42-8. PEB is a coproduct of ethylbenzene manufacture and a Class II complex mixture consisting of various isomers of alkylbenzene and diphenyl hydrocarbons.
Method/Guideline:	Calculated values using AOPWIN version 1.90, a subroutine of the computer program EPIWIN version 3.12 (U.S. EPA 2000) AOPWIN estimates the rate constant for the atmospheric, gas-phase reaction between photochemically-produced hydroxyl radicals and organic chemicals. The rate constants estimated by the program are then used to calculate atmospheric half-lives for organic compounds based upon average atmospheric concentrations of hydroxyl radicals.
GLP	Not Applicable
Year (study performed):	Not Applicable
Test Substance: **[components]**	Polyethylbenzene Bottoms (PEB, CAS RN 68987-42-8) is a co-product of ethylbenzene manufacture and a Class II complex mixture. It consists of various isomers of the following principal components. • Diphenylethanes • Diphenylmethanes • Triethylbenzenes • Diphenylpropanes
Type (air, soil, water, other):	Not Applicable
Light Source:	Sunlight
Light Spectrum: • Wave length value (upper/lower)	Natural sunlight
Relative Intensity:	1
Test Substance Spectrum:	Not Applicable
Test Conditions: • Note: Concentration, temperature, test system type, replication, deviations from guideline or protocol	Atmospheric oxidation potential is an indirect photodegradation process that is based on the structure-activity relationship (SAR) developed by R. Atkinson (1988, 1989). The SAR assumes the following conditions: Temperature: 25°C Sensitizer: OH- radical Concentration of Sensitizer: $1.5E^6$ OH- radicals/cm^3
Direct Photolysis: • Results: half-life, % degradation, quantum yield	Not Applicable

Indirect Photolysis: • Results: type of sensitizer, concentration of sensitizer, rate constant, % degradation, half-life	Calculated atmospheric oxidation potential (AOP) data for representative constituents of PEB are listed below. The data identify a potential AOP range for substances represented by the listed constituents. PEB does not have a specific atmospheric half-life; rather, actual half-life ranges for substances in this stream will vary dependent on their constituent composition. The compounds selected to represent the AOP range for PEB were selected on the basis of compositional analysis of a composite blend of streams from various suppliers. The following are AOP values calculated by the EPIWIN program: **Substance Constituent** / **Calculated half-life (day)** / **OH- Rate Constant (cm^3/molecule-sec)** Diethylbenzene / 1.3 / 8.1×10^{-12} 1,3,5-triethylbenzene / 0.32 / 1.4×10^{-11} 1,2,4-triethylbenzene / 0.60 / 3.3×10^{-11} Cyclohexylbenzene / 0.73 / 1.8×10^{-11} Diphenylmethane / 1.0 / 1.1×10^{-11} 1,1'-diphenylethane / 0.94 / 1.1×10^{-11} 1,2-diphenylethane (bibenzyl) / 0.89 / 1.2×10^{-11} 1,1'-diphenylpropane / 0.83 / 1.3×10^{-11}
Degradation Products: • Note: Identification, concentration	Unknown
Conclusion:	Atmospheric oxidation reactions from hydroxyl radical attack can significantly contribute to the degradation of constituent hydrocarbons in PEB. Constituent hydrocarbons have sufficiently high vapor pressures, indicating that such compounds will partition to air where oxidation reactions occur. Results from EQC Level 1 modeling of constituent hydrocarbons to assess environmental distribution support this evaluation. Based on calculated atmospheric oxidation potential values, hydrocarbons making up PEB have an atmospheric half-life range of approximately 0.3 to 1.3 days. These data suggest that the hydrocarbon constituents of this substance will degrade rapidly and not persist in the atmosphere.
Reliability:	**2.** Reliable with restrictions. Rate constants and half-lives presented in this robust summary were estimated using the AOPWIN program contained in the EPIWIN© model. They represent a potential range of atmospheric oxidation potentials based on constituent molecules in PEB.
References:	Atkinson, R. 1988. Estimation of gas-phase hydroxyl radical rate constants for organic chemicals. Environ. Toxicol. Chem. 7:435-442. Atkinson, R. 1989. Kinetics and mechanisms of the gas-phase reactions of the hydroxyl radical with organic compounds. J. Phys. Chem. Ref. Data Monograph No. 1, Amer. Inst. Physics & Amer. Chem. Soc., New York, NY, USA. U.S. EPA. 2000. Estimations Programs Interface for Windows (EPIWIN©). U.S. Environmental Protection Agency, Washington, DC.
Other (source): Last changed	1/31/06

Robust Summary - Polyethylbenzene Bottoms Stream

Stability in Water

Test Substance:	Polyethylbenzene Bottoms Stream (PEB) is 100% of the complex mixture CAS RN. 68987-42-8. PEB is a coproduct of ethylbenzene manufacture and a Class II complex mixture consisting of various isomers of alkylbenzene and diphenyl hydrocarbons.
Method/Guideline:	Other: Technical discussion
Type (test type):	Not Applicable
GLP	Not Applicable
Year (study performed):	Not Applicable
Analytical Monitoring:	Not Applicable
Test Conditions: • Note: Concentration preparation, vessel type, volume, replication, deviations from guideline or protocol	Not Applicable
Results: **Units/Value:** • Note: Analytical method, observations, half-lives by pH, degradation products	Not Applicable
Test Substance: **[components]**	Polyethylbenzene Bottoms (PEB, CAS RN 68987-42-8) is a co-product of ethylbenzene manufacture and a Class II complex mixture. It consists of various isomers of the following principal components. • Diphenylethanes • Diphenylmethanes • Triethylbenzenes • Diphenylpropanes
Conclusion:	<u>**Technical Summary**</u> Hydrolysis of an organic molecule occurs when a molecule (R-X) reacts with water (H_2O) to form a new carbon-oxygen bond after the carbon-X bond is cleaved (Harris, 1982; Neely, 1985) . This reaction is referred to as nucleophilic substitution, where X is the leaving group being replaced by the incoming nucleophilic oxygen from the water molecule. The leaving group, X, must be a molecule other than carbon because carbon atoms lacks sufficient electronegativity to serve as a good leaving group (i.e., carbon-carbon bonds are too stable to be cleaved by nucleophilic substitution). Chemicals that have a potential to hydrolyze include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters (Harris, 1982; Neely, 1985). The constituent compounds in PEB are hydrocarbons that contain only carbon and hydrogen. Thus, the PEB stream is not subject to hydrolysis,

	and this fate process will not contribute to the degradative loss of chemical constituents in this Class II complex mixture.
Reliability:	2. Reliable with restrictions. The technical summary presented herein was based on well-regarded scientific references.
Reference:	Harris, J.C. 1982. "Rate of Hydrolysis," Chapter 7 in: W.J. Lyman, W.F. Reehl, and D.H. Rosenblatt, eds., Handbook of Chemical Property Estimation Methods, McGraw-Hill Book Company, New York, NY, USA Neely, W. B. 1985. "Hydrolysis", Chapter 7 in: W. B. Neely and G. E. Blau, eds. Environmental Exposure from Chemicals. Vol I. 173. CRC Press, Boca Raton, FL, USA.
Other (source): Last changed	1/31/06

Robust Summary - Polyethylbenzene Bottoms Stream

Transport Between Environmental Compartments

Test Substance:	Polyethylbenzene Bottoms Stream (PEB) is 100% of the complex mixture CAS RN. 68987-42-8. PEB is a coproduct of ethylbenzene manufacture and a Class II complex mixture consisting of various isomers of alkylbenzene and diphenyl hydrocarbons.
Method/Guideline:	Calculated according to EQC Level 1 Model Version 2.02 (Trent University, 2003)
Type (test type):	Not Applicable
GLP:	Not Applicable
Year (study performed):	Not Applicable
Estimation Temperature:	25°C
Test Conditions: • Note: Concentration prep., vessel type, replication, test conditions.	The EQC model uses chemical-physical properties to quantify a chemical's behavior in an evaluative environment. It calculates the distribution of a fixed quantity of conserved (i.e., non-reacting) chemical, in a closed environment at equilibrium, with no degrading reactions, no advective processes, and no inter-media transport processes (e.g., no wet deposition, or sedimentation). The medium receiving the emission is unimportant because the chemical is assumed to become instantaneously distributed. Physicochemical input values (molecular weight, water solubility, vapor pressure, partition coefficient, and melting point) for the EQC model were obtained from EPIWIN (U.S. EPA, 2000) database. Measured values for input parameters were used when available; otherwise, modeled values were employed.
Results: **Units/Value:** • Note: Deviations from protocol or guideline, analytical method.	Calculated partitioning data for representative constituents of PEB are listed below. The range of distribution data for constituent chemicals in each of the compartments can be used as an estimate of the partitioning behavior for such streams.
Test Substance:	Polyethylbenzene Bottoms (PEB, CAS RN68987-42-8) is a co-product of ethylbenzene manufacture and a Class II complex mixture. It consists of various isomers of the following principal components. • Diphenylethanes • Diphenylmethanes

Substance Constituent	Air	Water	Soil	Sed.	Sus.Sed	Biota
	Calculated Percent Distribution					
Diethylbenzene	79.2	0.6	19.8	0.4	<0.1	<0.1
Cyclohexylbenzene	35.3	1.1	62.2	1.4	<0.1	<0.1
1,3,5-triethylbenzene	99.8	<0.1	0.2	<0.1	<0.1	<0.1
1,2,4-triethylbenzene	75.6	0.2	23.7	0.5	<0.1	<0.1
Diphenylmethane	16.3	6.2	75.8	1.7	<0.1	<0.1
1.1'-diphenylethane	28.5	5.2	64.9	1.4	<0.1	<0.1
1,2-diphenylethane	12.7	1.5	83.8	1.9	<0.1	<0.1
1,1'-diphenylpropane	11.9	2.2	84	1.9	<0.1	<0.1

	• Triethylbenzenes • Diphenylpropanes
Conclusion:	The partitioning data represent a potential distribution range for constituent hydrocarbon chemicals in PEB. These hydrocarbons were calculated to partition either to air or soil depending in large part on the number of ring constituents in the molecule. With the exception of cyclohexylbenzene, alkylbenzene constituents were shown to partition primarily to air and secondarily to soil. Cyclohexylbenzene and biphenyl compounds partitioned primarily to soil and secondarily to air. A small percentage of all compounds (<0.1 to 6.2%) partitions to water or sediment (<2%). The input data used to run the EQC Level I model preferentially used measured data from the EPIWIN database and estimated values calculated by the EPIWIN program based on chemical structure when measured data were not available.
Reliability:	**2.** Reliable with restrictions. The environmental distribution data presented in this robust summary were estimated using the EQC model developed by Trent University. They represent the potential environmental distribution of the test substance based on constituent molecules in PEB.
Reference:	Trent University. 2003. EQC Fugacity-Based EQC-Equilibrium Criterion Model. Canadian Environmental Modeling Centre, Trent University, Peterborough, Ontario (http://www.trentu.ca/cemc/). U.S. EPA. 2000. Estimations Programs Interface for Windows (EPIWIN). U.S. Environmental Protection Agency, Washington, DC.
Other (source): Last changed	 1/31/06

Robust Summary - Polyethylbenzene Bottoms Stream

Invertebrate Acute Toxicity

Test Substance	Polyethylbenzene Bottoms Stream (PEB) is 100% of the complex mixture CAS RN. 68987-42-8. PEB is a coproduct of ethylbenzene manufacture and a Class II complex mixture consisting of various isomers of alkylbenzene and diphenyl hydrocarbons.
Method/guideline	OECD Guideline 202, Part 1 (1992)
Type (test type)	Static-renewal, water accommodated fraction
GLP	yes
Year (study performed)	2005
Species	*Daphnia magna*
Analytical Monitoring	yes
Exposure Period	48 hours
Statistical Methods:	EC50 by the Trimmed Spearman-Karber Method
Test Conditions: Note: concentration prep., vessel type, volume, replication, water quality parameters, environmental conditions, supplier of organisms, age, size, loading	The PEB sample was a blend of equal volumes of six PEB samples from different suppliers.Groups of *Daphnia magna* were exposed to a negative control, a solvent control (0.05 mL acetone/L) and five concentrations of the test substance and assessed for immobilization for 48 hours. Exposure solutions were prepared as water accommodated fractions (WAFs) of Polyethylbenze Bottoms (PEB) blend, and exposure solutions were renewed at 24 hours using fresh WAFs. The experimental treatments included control, solvent control, and five PEB loading rates of 65, 130, 250, 500, and 1000 µg/L. WAFs were prepared by adding appropriate volumes from five stock solutions of the test substance to 2.0 L of dilution water in each of five 2.0-L glass aspirator bottles. Each bottle was sealed with parafilm and stirred with a teflon stir bar for approximately 2 hours. Stirring speed was adjusted to create a slight vortex in each bottle (<25% of the solution depth). Once the stirring period ended, the liquid phases in the bottles were allowed to separate for approximately 30 min. Control (dilution water) and solvent control (0.05 mL acetone/L) solutions were treated in the same manner. From each aspirator bottle, solution was drained from the bottom outlet into four replicate 8-oz (237-mL) glass jars, which served as test vessels. Vessels were completely filled and sealed with a glass plate to eliminate all headspace. Remaining solution from each aspirator bottle was used for water quality measurements and analysis for the test substance. Dilution water used in testing and culturing daphnids was aged laboratory freshwater prepared by blending naturally hard well water with well water that was de-mineralized by reverse osmosis. The waters were blended to yield a total hardness of 130 to 160 mg/L as $CaCO_3$ and biologically aged. First-instar neonates, less than 24 hours old were used to initiate the test. Neonate daphnids originated from cultures maintained in the testing laboratory, where adults were fed at least once a day a suspension of the alga, *Pseudokirchneriella subcapitata*, supplemented by a prepared artificial invertebrate food. Daphnids used in testing were not fed. Adults that produced the young were approximately 18 days old and showed no signs of stress or physical damage. Five daphnids were randomly assigned and carefully transferred to each of four replicate test vessels, giving a total of 20 daphnids for each experimental group.

	Vessels were place in a 20 ± 1°C temperature-controlled waterbath. Lighting was provide by fluorescent bulbs at an intensity of 502 lux at the level of the test vessels. A photoperiod of 16-hour light and 8-hour dark with a 30 min dusk/dawn transition period was used during the test. Numbers of immobilized daphnids were recorded at 24 and 48 hours. The concentrations of PEB in the WAF solutions were measured in samples collected at 0 hour (fresh solutions), 24 hours (fresh and old solutions), and 48 hours (old solutions). Analyses were done using gas chromatography with a flame ionization detector. Responses of standards and samples were calculated as the sum of the responses from six marker peaks within the PEB chromatogram. Temperature measurements of the exposure solutions during the test ranged from 19.0 °C to 19.8 °C, dissolved oxygen ranged from 8.0 mg/L to 8.7 mg/L, and the pH in all solutions was 8.3 for the duration of testing. Hardness, alkalinity, and specific conductance of the dilution water at test initiation were 412 mg/L as $CaCO_3$, 156 mg/L as $CaCO_3$, and 347 µS, respectively. Measured concentrations of PEB in WAF solutions were:

Nominal Loading Rate µg/L	0-hr fresh	24-hr old	24-hr fresh	48-hr old	mean	% nominal
Control	<MQL	< MQL	< MQL	< MQL	< MQL	--
Solv. Control	< MQL	< MQL	< MQL	< MQL	< MQL	--
65	60.6	60.4	61.9	61.1	61	94
130	120	110	131	105	117	90
250	237	233	234	122	207	83
500	464	452	466	468	463	93
1000	918	868	977	842	901	90

Minimal Quantifiable Limits [MQL] = 41.6 µg/L

Results Units/Value: Note: Deviations from protocol or guidelines, analytical method, biological observations, control survival	24-hour EC50 = >1000 µg/L, based on nominal WAF loading rates 48-hour EC50 = 340 µg/L, based on nominal WAF loading rates. 95% confidence limits = 310 µg/L and 310 µg/L. 48-hour No-Observed-Effect Concentration = 130 µg/L The slope of the dose-response line at 48-hours was 9.7. The following dose response at 48 hours was obtained in the test.

Nominal Loading Rate, µg/L	48-hour % Immobilized
Control	0
Solvent control	0
65	0
130	0
250	10
500	95
1000	95

There were no deviations from the protocol or guideline.

Conclusions (Laboratory contractor)	24-hour EC50 = >1000 µg/L based on nominal WAF loading rates 48-hour EC50 = 340 µg/L based on nominal WAF loading rates. 48-hour NOEC = 130 µg/L The 48-hour dose-response slope = 9.7

Reliability	**1.** Reliable without restrictions
Reference	Analytical Bio-Chemistry Laboratories (ABC). 2005. Acute toxicity of polyethylbenzene bottoms stream blend (PEB) to the water flea, Daphnia magna, determined under static-renewal test conditions. ABC Study No. 49029, ABC Laboratories, Columbia, MO. Sponsor: American Chemistry Council, Arlington, VA
Other Last changed	 1/31/06

Robust Summary – Polyethylbenzene Bottoms Stream

Genetic Toxicity –In Vitro

Test Substance:	Polyethylbenzene Bottoms Stream (PEB) is 100% of the complex mixture CAS RN. 68987-42-8. PEB is a coproduct of ethylbenzene manufacture and a Class II complex mixture consisting of various isomers of alkylbenzene and diphenyl hydrocarbons.
Method/Guideline:	OECD Guideline 471 (1998)
Type (test type):	Bacterial Reverse Mutation Assay
System of testing	*Salmonella typhimurium, E. coli*: plate incorporation ±S9
GLP:	Yes
Year (study performed):	2005
Species/Strain	*Sal. typhimurium* strains TA 1535, 1537, 100, 98 and *E. coli* WP2uvrA
Metabolic activation	Yes
Species and Cell type	Sprague Dawley rat liver homogenate (S9)
Quantity	10% homogenate in S9 mix
Induced or not induced	Livers from rats induced with Aroclor 1254 by single 500mg/kg IP injection, 5 days prior to sacrifice
Concentrations tested	0, 1.5 to 5000µg/plate in several assays
Statistical Methods:	Not applicable. Criteria for positive response are a dose-related increase in mean revertants per plate in at least one tester strain over a minimum of 2 increasing concentrations. Results were positive for TA1535 and TA1537 if the peak of the dose response was ≥ 3-fold the mean vehicle control value; for TA100, TA98 and *E. coli* WP2 uvrA if the peak of the dose response was ≥ 2-fold the mean vehicle control value.
Test Conditions	The PEB sample was a blend of equal volumes of six PEB samples from different suppliers. PEB diluted in ethanol (EtOH) was tested in 4 strains of *Salmonella* and *E. coli* WP2 uvrA with and without S9 metabolic activation in an initial toxicity/mutagenicity test (2 plates/dose) and 3 confirmatory mutagenicity assays (3 plates/dose). Doses of PEB solubilized in EtOH formed a clear, soluble solution at 500mg/ml, the highest concentration prepared. In the initial toxicity/mutagenicity trial (B1) - all *Salmonella* strains and *E. coli*, ±S9, doses were 0, 1.5, 5.0, 15, 50, 150, 500, 1500, 5000µg/plate. In the first mutagenicity trial (B2) – all *Salmonella* strains and *E. coli* ±S9, doses were 0, 15, 50, 150, 500, 1500, 5000µg/plate. The next trial (B3) was aborted due to unacceptable vehicle controls. Confirmatory trial B4 tested TA 98±S9 at doses of 0, 15, 50, 150, 500, 1500, 5000ug/plate and TA100 +S9 at 0, 50, 150, 500, 1500, 2000, 3000, 5000ug/plate. To verify mutagenic activity seen with TA100+S9, trial B5 was performed in TA100 ±S9 at doses of 0, 50, 150, 500, 1500, 2000, 3000, 5000µg/plate. Two other repeat assays using TA100 and PEB demonstrated severe toxicity over a range of doses without evidence of mutagenicity and were not considered definitive for this assay. In all assays 50µl PEB in ethanol at appropriate concentrations or vehicle was introduced into molten minimal top agar (45± 2^0C), along with 100µl of bacterial tester strain (10^9 cells/ml), 0.5ml of S9 mix or sham mix, blended by vortexing, and poured onto the surface of a 25ml

	solid minimal bottom agar plate. When top agar had set, plates were inverted and incubated for 48-72hrs at 37±2^{0}C. At the end of incubation, plates were evaluated for toxicity to background lawn and revertant colonies were counted. Replica plating was performed as appropriate to verify presence of mutant colonies from the original test plate. Positive control compounds for assays were 2-amino anthracene for +S9 plates for all *Salmonella* strains and *E. coli*; for –S9 plates, TA98, 2-nitrofluorene; TA100 and TA1535, sodium azide; TA1537, 9-aminoacridine; *E. coli* WP2 uvrA, methyl methane sulfonate.
Results: Genotoxic effects	In the initial toxicity/mutagenicity trial (B1), toxicity [reduction] to background lawn was visible at 5000µg/plate in all *Salmonella* strains and observed as a slight reduction in lawn at ≥ 500 or ≥ 1500µg/plate depending on strain and precipitate was observed beginning at 1500µg/plate ±S9 in all strains. In *E. coli*, no lawn reduction was seen and precipitate was observed beginning at 1500 µg/plate ±S. TA100 demonstrated a positive mutagenic response of 2.3 fold maximum increase above controls with S9 and 2.1 fold increase above controls without S9 at 5000ug/plate. No other *Salmonella* strain or *E. coli* showed revertant numbers in excess of negative control values. In mutagenicity trial B2 using all strains ±S9 no positive response was observed in any strain. Toxicity was observed beginning at 500 or 1500µg/plate depending on the strain and precipitate was seen beginning at 500 or 1500µg/plate. Slight reduction in background lawn was observed at 5000µg/plate in *E. coli* ±S9. Trial B4 was performed with TA100+ S9 due to severe toxicity at 1500 and 5000µg/plate not seen in TA100-S9, and with TA98±S9 due to numerous microcolonies that obscured accurate counting in the previous trial. In this test, TA98 did not demonstrate any increases in mutant colonies above controls at any dose level ±S9. TA100+S9 showed a positive response with 2.1 to 2.4-fold increases above control values at 1500, 2000, 3000 and 5000µg/plate. To confirm the mutagenic activity in TA100, trial B5 was performed with TA100 ±S9. No increase in revertant colonies of 2-fold or greater was seen with TA100-S9. The weak positive response seen with TA100 – S9 in trial B1 was not reproduced in trials B2 or B5. TA100+S9 again demonstrated a positive mutagenic response of 2.2 to 2.9-fold increase over negative control values at 1500, 2000, 3000 and 5000µg/plate. All positive control compounds demonstrated appropriate mutagenic activity in all assays.
Conclusion: (Laboratory contractor)	PEB induced a positive repeatable mutagenic response in *Salmonella typhimurium* TA100 with metabolic activation. The increase did not exceed 2.9 fold of negative controls in any trial. No other *Salmonella* strain or *E. coli* demonstrated mutagenic activity. PEB is a bacterial gene mutagen in this test system.
Reliability	1. Reliable without restriction
Reference	Bacterial Reverse Mutation Assay – Polyethylbenzene Bottom Stream (PEB), CAS No. 68987-42-8. 2005. San, R.H.C. and Klug, M.L. [AB00CN.503.BTL; Sponsor Project No. WIL-186036] BioReliance, Rockville, MD. Sponsor: American Chemistry Council, Arlington, VA.
Other (source) Last changed	1/31/06

Robust Summary – Polyethylbenzene Bottoms Stream

Genetic Toxicity –In Vitro

Test Substance:	Polyethylbenzene Bottoms Stream (PEB) is 100% of the complex mixture CAS RN. 68987-42-8. PEB is a coproduct of ethylbenzene manufacture and a Class II complex mixture consisting of various isomers of alkylbenzene and diphenyl hydrocarbons.
Method/Guideline:	OECD Guideline 473 (1998)
Type (test type):	Mammalian cell Chromosome Aberration test
System of testing	Rodent cells in culture
GLP:	Yes
Year (study performed):	2005
Species/Strain	Chinese Hamster Ovary (CHO) cells
Metabolic activation	Yes
Species and Cell type	Sprague Dawley rat liver homogenate (S9)
Quantity	20µl S9/ml McCoy's 5A culture medium
Induced or not induced	Livers from rats induced with Aroclor 1254 by single 500mg/kg IP injection, 5 days prior to sacrifice
Concentrations tested	Preliminary toxicity: 0, 15 to 5000µg/ml; Chromosome assay: 0, 3.13 to 150µg/ml. Analyzed doses: 0. 6.25, 12.5 and 25.0µg/ml
Statistical Methods:	Percent of aberrant cells analyzed by Fisher's exact test (p=0.05), then Cochran-Armitage to measure dose responsiveness.
Test Conditions	The PEB sample was a blend of equal volumes of six PEB samples from different suppliers. PEB diluted in ethanol (EtOH) was administered to CHO cells (5 x 10^5 cells/ 25cm^2 flask) ±S9 to determine possible induction of chromosome damage in cultured mammalian cells. Cells were seeded in flasks containing McCoy's 5A medium supplemented with 10% fetal bovine serum, antibiotics and L-glutamine. For testing, cells were refed with S9 reaction mixture [S9 homogenate + co-factors] at 1ml volume in 4ml serum-free medium or with 5ml complete medium for non-activated assays, as appropriate. Test article or solvent was then added at 50µl. Osmolality in treatment medium with solvent, highest PEB concentration, lowest PEB concentrations causing precipitate or highest soluble PEB concentration was measured. The pH of the highest concentration of dosing solution in medium was also determined with pH test tape. <u>Preliminary Toxicity assay</u>: CHO cells were exposed to EtOH (solvent-negative control) or 9 concentrations of PEB ±S9 for 4 hrs, or without S9 for 20hrs continuously. Cells were incubated at 37±1^{0}C in a humidified atmosphere of 5±1% CO_2 in air. After the 4 hr exposure, cells were washed, resuspended in complete medium and incubated for a total of 20 hrs from initiation of treatment. After 20 hrs, cells were harvested, trypsinized and counted using a Coulter Counter. Cell viability was determined by trypan blue dye exclusion. <u>Chromosome Aberration test</u>: Duplicate cultures of CHO cells were exposed to PEB ±S9. In the initial and repeat non-activated assays, cells were exposed for 4 hr or 20 hr continuously at 37±1^{0}C and all cultures were incubated for 20hr total.

	Two hours prior to harvest, cells were treated with Colcemid® at a final concentrations of 0.1µg/ml medium. In the initial and repeat S9-activated assays, cells were exposed to PEB for 4 hrs, treatment medium was removed, cells were washed, refed and incubated for a total of 20hrs. Positive control compounds were mitomycin C [0.1 and 0.2 µg/ml] for non-activated cultures and cyclophosphamide [10 and 20µg/ml] for activated cultures. A concurrent toxicity test ±S9 was performed using an aliquot of cell suspension from each culture flask collected at cell harvest, to determine cell growth inhibition. At harvest, cells were collected by trypsinization and centrifugation at 800rpm for 5 min. Cell pellet was resuspended in 2-4ml 0.075M KCl and allowed to stand at room temperature for 4-8 min. Cells were recentrifuged, supernatant aspirated and cells fixed with 2 washes of 2ml Carnoy's fixative (methanol:glacial acetic acid, 3:1, v/v) Cells were stored overnight in fixative at approx. 2-8^{0}C. In the morning, cells were centrifuged at 800rpm for 5 min and medium changed twice; after decanting the second fixative supernatant, cells were resuspended to opalescence in fresh fixative and a small aliquot was dropped onto the center of a clean glass slide and allowed to air dry. Slides were stained with 5% Giemsa, air dried and permanently mounted. Slides were identified by study number, date and treatment condition. Analysis: The highest dose level selected for analysis of chromosome aberrations was the dose that induced at least 50% toxicity as measured by mitotic inhibition relative to solvent controls with a sufficient number of scorable metaphase cells. Two additional lower dose levels were also evaluated. Slides were coded using random numbers by an individual not involved with the study and evaluated "blind" by the cytogeneticist. A minimum of 200 metaphase spreads [100 per duplicate flask] were scored for chromatid and chromosome-type aberrations. Pulverized chromosomes and severely damaged cells (≥10 aberrations) were recorded. Numerical aberrations (polyploidy and endoreduplication) were also recorded. Chromatid gaps were recorded but not included in the analysis.
Results: Genotoxic effects	Preliminary Toxicity: Dose levels for the chromosome aberration assay were selected following a preliminary toxicity test based on reduction in cell growth relative to solvent control. Visible precipitate was observed at dose levels ≥ 150µg/ml, dose levels ≤ 50µg/ml were soluble in treatment medium at the beginning and conclusion of the treatment period. Osmolalities for treatment groups were within 2-3% of solvent control, pH = 7.0 in all treated flasks. Substantial toxicity occurred at ≥ 150µg/ml in non-activated 4 and 20 hr exposure groups and at levels ≥ 50µg/ml in S9 activated 4 hr exposure groups. Chromosome aberration assay: Dose levels selected for all treatment regimens were 0, 3.13, 6.25, 12.5, 25, 50, 75, 100, 125 and 150µg/ml. Visible precipitate was observed at dose levels ≥ 100µg/ml; dose levels ≤ 75µg/ml were soluble in treatment medium and the beginning and conclusion of treatment. Osmolality and pH of treated cultures were comparable to controls. 4hr exposure –S9: Dose levels evaluated were 6.25, 12.5, and 25µg/ml. Mitotic Index at 25µg/ml was reduced 52% relative to solvent controls. The percentage of cells with numerical or structural anomalies was not significantly increased above solvent control values at any dose level. 4hr exposure +S9: Dose levels evaluated were 3.13, 6.25, and 12.5µg/ml. Mitotic Index at 12.5µg/ml was reduced 53% relative to solvent controls. The percentage of cells with numerical or structural anomalies was not significantly increased above solvent control values at any dose level. 20hr exposure –S9: Dose levels evaluated were 6.25, 12.5, and 25µg/ml. Mitotic

	Index at 25µg/ml was reduced 54% relative to solvent controls. The percentage of cells with structural anomalies was not significantly increased above solvent control values at any dose level. The percentage of cells with numerical aberrations (polyploidy and/or endoreduplication) was statistically significantly increased at dose levels of 12.5 and 25µg/ml [$p \leq 0.05$, Fischer's Exact test] but no dose response was seen in the Cochran-Armitage test. Since the percentage of cells with numerical aberrations at dose levels 12.5 (7.5%) and 25ug/ml (7.0%) were within the historical control range of 0.0 to 7.5% for this laboratory and there was no increasing dose response, this effect was not considered biologically significant. Confirmatory test for absence of effect with metabolic activation: A repeat test was performed with a 4 hr exposure +S9. Dose levels evaluated were 6.25, 12.5, and 25µg/ml. Mitotic Index at 25µg/ml was reduced 53% relative to solvent controls. The percentage of cells with numerical or structural anomalies were not significantly increased above solvent control values at any dose level. Positive control compounds in all assays demonstrated appropriate clastogenic activity.
Conclusion: (Laboratory contractor)	PEB is not clastogenic to mammalian cells in culture. No biologically significant increases in structural or numerical aberrations were observed in chromosomes at any dose levels in any exposure regimen.
Reliability	1. Reliable without restriction
Reference	*In Vitro* Chromosome Aberration Test – Polyethylbenzene Bottom Stream (PEB), CAS No. 68987-42-8. 2005. Gudi, R., and Rao, M. [AB00CN.331.BTL; Sponsor Project No. WIL-186037] BioReliance, Rockville, MD. Sponsor: American Chemistry Council, Arlington, VA.
Other (source) Last changed	1/31/06

Robust Summary – Polyethylbenzene Bottoms Stream

Repeated Dose Toxicity Study with Reproductive/Developmental Screening

Test Substance:	Polyethylbenzene Bottoms Stream (PEB) is 100% of the complex mixture CAS RN. 68987-42-8. PEB is a coproduct of ethylbenzene manufacture and a Class II complex mixture consisting of various isomers of alkylbenzene and diphenyl hydrocarbons.
Method/Guideline:	OECD Guideline 422 (1996)
Type (test type):	28 day repeated dose oral toxicity study with neurobehavioral endpoints and reproductive/developmental screening
GLP:	Yes
Year (study performed):	2005
Species/Strain	Rats – Sprague Dawley
Route of Administration	Oral gavage
Duration of Test	Approximately 8 weeks
Doses/concentration levels	0, 20, 80, and 320 mg/kg/day
Sex	12 males and 12 females/group
Exposure period	Males 35-37 days; Females, max. 52 days [2 wks premating, 2 wks mating, gestation days (GD) 0-21 to lactation days (LD) 3-4.
Frequency of Treatment	Once/day, 7 days/week
Control group and Treatment	12 males, 12 females Corn oil, 5ml/kg/day, 7 days/wk
Statistical Methods:	2-tailed tests at 1 and 5% significance levels. Litter was experimental unit as appropriate. Data from non-gravid females excluded following mating period. Chi square was used for mating, fertility, conception and copulation indices. Parametric one-way analysis of variance (ANOVA) for body wt and wt gains [parents and offspring] food consumption, number of pups, live litter size at postnatal day (PND) 0, unaccounted for sites, clinical pathology, absolute and relative organ wt, precoital intervals, Functional Observational Battery (FOB) data. If intergroup variances were seen, Dunnett's test used for comparisons between groups. Kruskal Wallis nonparametric ANOVA was used for percentage of males/litter at birth, postnatal survival, then Dunn's test was used for group comparisons. FOB parameters yielding scalar or descriptive data were analyzed by Fisher's exact test. Locomotor activity parameters were analyzed by repeated measure analysis of variance (RANOVA). Sequential linear trend tests were used for monotonic dose response relationships. Non-monotonic trends, evaluated whenever no significant linear trends were detected by treatment (TRT) and/or the TRT*TIME interaction was significant at the 0.01 level, were analyzed within the RANOVA pair-wise comparison package. Total count locomotor activity data were analyzed at BioSTAT Consultants, Inc., Portage, MI. Ambulatory counts were subjected to one-way ANOVA then Dunnett's if appropriate.
Test Conditions	The PEB sample was a blend of equal volumes of six PEB samples from different

	suppliers. Sprague Dawley rats (56 days of age) were received from Charles River Laboratories, Raleigh, NC and acclimated for 16 days. Twelve males (322.8 – 390.8g, 10 wks of age) and 12 females (201.5 – 258.8g, 10 wks of age) were assigned to each treatment group. PEB in corn oil was administered in doses of 0, 20, 80 and 320mg/kg once daily by oral gavage, 7 days/wk. Males were treated from 14 days prior to mating to 1 day prior to sacrifice or on the day of sacrifice for males assessed for neurobehavioral parameters for a total of 37-39 days. Females were treated from 14 days prior to mating through gestation to lactation day (LD) 3 or 4 if assessed for neurobehavioral parameters for a total of 39 (non-mated females) to 52 doses. Animals were housed in individual stainless steel wire mesh cages until mating, then paired 1:1 in the male's home cage. Following copulation confirmed by vaginal plug or sperm in vaginal lavage sample, designated gestation day (GD) 0, females were transferred to plastic boxes with ground corncob bedding (Bed-O'Cobs® - analysis from manufacturer) as nesting material. Females remained housed in these boxes until sacrifice at LD 4. Food and water was available *ad libitum*. Room conditions were 22±3^{0}C average temperature, 50±20% humidity with a 12-hour light/dark cycle and 12 air changes/hr. <u>Analysis</u>: Dosing solutions were prepared weekly. Dosing solutions were evaluated for homogeneity, resuspension homogeneity, and stability prior to study initiation and samples were taken during the study to verify concentrations at each dose level for the first two weeks of administration and monthly thereafter. Four major peaks areas were identified for PEB in corn oil by gas chromatographic analysis at retention times of 8.0, 9.6, 10.0 and 10.2 minutes. Concentrations were back calculated from results of regression analysis of the sum of these 4 major peaks. A Certificate of Analysis of the major components of PEB was supplied with this study. <u>Clinical Observations</u>: All rats were observed twice daily for moribundity and mortality. Clinical observations were recorded daily. Once prior to study initiation and weekly thereafter, rats were observed outside the home cage for behavioral changes. Animals were observed at dosing and 1 hour after dosing for signs of overt toxicity. <u>Body weights and Food consumption</u>: Body wt data were recorded weekly for males and females until beginning of gestation. Thereafter female body wts were recorded at GD 0, 4, 7, 11, 14, 17, 20 and LD 1 and 4. Weights of non-pregnant females were recorded weekly. Food consumption was recorded over the same intervals except during mating. <u>Parturition</u>: Pregnant rats were observed twice daily for initiation and completion of parturition and signs of dystocia. On postnatal day 0 pups were sexed and examined for malformations, and the number of stillborn and live pups were recorded. Gestation length was calculated from the date at which parturition began. <u>Neurobehavioral Parameters</u>: FOB [Functional Observational Battery] observations were recorded for 6 rats/sex/group during week 5 (males) and on LD 4 (females) approximately 1 hour postdose. Testing was performed by the same technicians without knowledge of group assignment in a sound-attenuated room with a white noise generator set at 70±10dB. Observations included home cage and handling, open field, sensory (e.g. startle response, forelimb and hindlimb extension, air righting reflex, tail pinch), neuromuscular observations (e.g. hindlimb foot splay, fore and hindlimb grip strength, rotarod performance), and physiological observations (catalepsy, body wt, body temperature). Locomotor activity was recorded after completion of FOB using a photobeam activity system. Data were collected in 5-minute epochs for a test duration of 60

	minutes. Total motor activity was a combination of fine motor skills (i.e. grooming, interruption of one photobeam) and ambulatory motor activity (interruption of 2 or more consecutive photobeams). <u>Clinical Pathology</u>: Blood samples were collected for hematology and serum chemistry from non-fasted rats, 6/sex/group at scheduled necropsies; study week 5 for males and LD 4 for females. <u>Necropsy</u>: Males were sacrificed following completion of the mating period (approx. wk 5). Females that delivered were sacrificed on LD4, and the number of former implantation sites and corpora lutea were recorded. Females that failed to deliver were sacrificed on postmating day 25 (females with evidence of mating) or post-cohabitation day 25 (females without evidence of mating). Uteri were stained with 10% ammonium sulfide for detection of early implantation loss. Females with total litter loss were sacrificed within 24 hrs of total loss. The following organs were weighed for all parental animals: adrenal glands, brain, heart, kidneys, liver, lungs, spleen, thymus, thyroids with parathyroids, testes, epididymides, prostate, ovaries with oviduct and uterus. Thirty-nine tissues and all gross lesions were collected and fixed in 10% neutral-buffered formalin, except for testes, which were fixed in Bouin's solution. <u>Histopathology</u>: Slides were prepared for protocol specified tissues and stained with hematoxylin-eosin, except for testes, which were stained with PAS. Microscopic evaluation was performed on all tissues from the control and 320mg/kg/day groups and on kidney, liver and thyroid glands in males and thyroid and thymus glands from females in the 20 and 80mg/kg/day groups. <u>F1 Litter observations</u>: Each litter was examined daily for survival. Pups were individually identified by digit tattoo. Intact offspring that died were necropsied using a fresh dissection technique including heart and major vessels. Each living pup was examined, sexed and weighed on LD1 and 4, and monitored for abnormalities in nursing behavior. Mean pup weights were presented by sex for each litter and by dose group. Litter parameter calculations included mean litter size, postnatal survival between birth and postnatal day 0 or birth and postnatal day 4 as percentage of litters, and % litters postnatal survival for all other intervals (PND0-1 and 1-4).
Results: NOAEL (NOEL) LOAEL (LOEL) Remarks	Parental systemic NOAEL = 20mg/kg/day Reproductive NOAEL = 20mg/kg/day Neonatal toxicity NOAEL = 320mg/kg/day Parental systemic LOAEL = 80mg/kg/day [decreased body wt and/or food consumption, organ wt changes and microscopic findings in 320mg/kg/day organs] Reproductive LOAEL = 80mg/kg/day [extended gestation, decreased number of implantations and pups born, and decreased live litter size] <u>Test material</u>: PEB test formulations were homogeneous and contained the appropriate concentrations. Each batch of test material was stable for at least 8 days. <u>Clinical Observations</u>: All rats survived to scheduled necropsy. Increased incidence of hair loss on the ventral abdomen and/or hindlimb at daily examinations, excessive pawing of cage surfaces at time of dosing, clear or red material on body surfaces 1 hr after dosing were seen in 320mg/kg/day animals. Increased incidence of clear and/or red material around the mouth was also seen in 80mg/kg/day group females 1 hr after dosing. Clear or red material was

considered to be due to potential taste aversion to the test article and not a sign of toxicity. The finding seen shortly after dosing did not persist to the next observation point. No clinical findings were observed in 20mg/kg/day rats.

Body weights and Food Consumption: Mean body wt, weight gain and/or food consumption in the 80 and 320mg/kg/day group males were reduced generally throughout the study. No effects were observed in females during the pre-mating period. During gestation and lactation, mean body wt and/or weight gain were reduced in 320mg/kg/day females; no effects in groups 80 or 20mg/kg/day females. Mean food consumption in all groups of females during gestation and lactation were comparable to controls.

Neurobehavioral Parameters: No significant PEB related effects on FOB parameters or locomotor activity were observed in males during study wk 5 or females on LD 4. A statistically significant ($p<0.05$) decrease in rotarod performance in 320mg/kg/day females [59.8 ± 51.22 sec.] compared to controls [111.1 ± 21.8 sec.] was attributed to biological variation and small sample size. Only 2/6 320mg/kg/day females [remained on rotarod for <30 sec] were affected and control performance was exceptionally high [5/6 female rats remained on the rod for the entire 120 second testing period]. Historical control data for rotarod performance at the testing laboratory is approximately 86.5 ± 49.07sec. for males and 76.1± 42.37 sec. for females.

Clinical Pathology: Statistically significant decreases in mean absolute and/or % eosinophils were observed in 80 mg/kg/day males and 320mg/kg/day animals of both sexes. No other hematology finding were observed; serum chemistry parameters were unaffected by treatment at all dose levels.

Necropsy and Pathology: Mean absolute and relative kidney weights in 80 and 320mg/kg/day males were increased and correlated with mineralization, multifocal deposits and irregular basophilic material in kidneys of 320mg/kg/day males examined microscopically. Mean absolute and relative liver wts were increased in 320mg/kg/day males and females that correlated with hepatocellular hypertrophy observed microscopically. Follicular cell hypertrophy was observed in thyroid gland of 320mg/kg males and females, which correlated with increased thyroid gland wt in females of this group. Atrophy of the thymus was observed in 3 females in the 320mg/kg/day group correlating with decreased thymus weight in these animals but no atrophy was seen in male thymus although thymus weight was decreased in these rats. No PEB related microscopic findings were seen in organs examined from 20 or 80 mg/kg/day group animals.

Reproduction Parameters: No effects were observed on male and female mating, fertility and copulation/conception indices. Mean number of days to mating were unaffected by PEB treatment. Mean gestation length in the 320mg/kg/day female (22.6 days) was statistically significantly increased ($p<0.01$) compared to controls (21.6 days). One female in this group delivered a single pup on gestation day 24 that was found dead on the day of delivery. At necropsy, the mean number of implantation sites was decreased in 80 and 320mg/kg/day groups, 14.6 and 14.4 /dam, respectively compared to controls (16.0/dam). However, since the decrease in the 80mg/kg/day group was due to one female with only 8 implantation sites, the effect was attributed to biological variation in this group. The mean number of unaccounted for sites was increased in the 320mg/kg/day group (2.2/dam) compared to control (1.0/dam). The mean number of pups born and live litter sizes on postnatal day 0 were reduced in the 80 and 320mg/kg/day groups. Values for the 80mg/kg/day group were 13.3 mean pups born and 13.3 mean live litter size [10 litters], and for the 320mg/kg/day group were 12.2 mean pups born and 11.9 mean live litter size [11 litters] compared to control values of 15.0 mean pups born and 15.0 mean live

	litter size [11 litters]. None of these findings was statistically significant. F1 Litter: No PEB related effect on the percentage of males at birth or postnatal survival was noted at any dose level. The general physical condition and mean pup body weights were unaffected by PEB treatment of parental animals at any dose level. There were no PEB-related findings on pups found dead or at scheduled necropsy on postnatal day 4.
Conclusions: (Laboratory contractor)	PEB induced both parental systemic toxicity and some evidence of reproductive toxicity in treated rats. Systemic toxicity was expressed as decrements in body weight and weight gain, some decreased food consumption and changes in organ weights at 80 and 320mg/kg/day groups with correlative microscopic findings in 320mg/kg/day animals. Reproductive changes included extended mean gestation length in 320mg/kg/day females and observed decreases in implantation sites, numbers of pups born and live litter size in 80 and 320mg/kg/day groups and increased unaccounted for sites at 320mg/kg/day. Although the changes in implantation sites, unaccounted for sites, pups born and live litter size were not statistically significant, these dose related occurrences were considered biologically significant for this screening test.
Reliability:	1. Reliable without restriction
Reference:	A Combined 28-day Repeated Dose Oral Toxicity Study with the Reproductive/Developmental Toxicity Screening Test of Polyethylbenzene Bottoms Stream (PEB) in Rats. 2005. Wilson, D.T. and Nemec, M. (Study No. WIL-186034). WIL Research Laboratories, LLC, Ashland OH. Sponsor: American Chemistry Council, Arlington, VA
Other (source) Last changed	 1/31/06